CHROMOSOME MAPPING IN SACCHAROMYCES: CENTROMERE-LINKED GENES[1,2]

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AS organisms for genetic studies, yeast have certain advantages which include: a short generation time, several relatively stable states of ploidy, an ease of handling which permits the application of many of the techniques employed with bacteria, the possibility of a wide variety of biochemical studies, and the feasibility of tetrad analysis. To make use of these characteristics in certain types of experiments, chromosome maps are needed. This paper deals principally with one aspect of a wider program of chromosome mapping of Saccharomyces, the use of centromere-linked genes to estimate the chromosome number and provide a coherent framework for future studies of linkage.

MATERIALS AND METHODS

Of the 140 genes now identified in our yeast stocks, we have restricted ourselves to those loci which show centromere linkage and to a few others which are common to several breeding stocks and are of possible interest to other workers in the field of yeast genetics. They are listed in Table 1, together with their source, when appropriate, and with references. More complete characterizations of the phenotypes for the genes controlling the fermentation of the sugars are given by WINGE and ROBERTS (1948); LINDEGREN (1949); DOUGLAS and CONDIE (1954); and HAWTHORNE (1955a). CHEN, EPHRUSSI and HOTTINGUER (1950) described the original genic petite strain. A phenotype similar to film formation was described by MAGNI (1949). The isolation of the nutritional mutants is reported by EPHRUSSI, HOTTINGUER and TAVLITSKI (1949); POMPER and BURKHOLDER (1949); REAUME and TATUM (1949); and MORTIMER, LERNER and BARR (1957).

The strains used were heterothallic. Diploid hybrid clones were obtained by pairing haploid cells (CHEN 1950), by isolating single zygotes from a mating mixture, or by the prototroph-selection technique (POMPER and BURKHOLDER 1949). Originally, the gypsum slant technique described by LINDEGREN and LINDEGREN (1944) was used to promote spore formation, and only linear four-

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TABLE 1

The sources of the genes included in this report

Gene symbol	Phenotype	Strain	Received from	Reference
a	Mating type	93–3P	C. C. LINDEGREN	LINDEGREN 1949
α	Mating type	93–1C	C. C. LINDEGREN	LINDEGREN 1949
ad_1	Adenine requiring (pink)	99R	E. L. TATUM	REAUME and TATUM 1949; LINDEGREN 1949; ROMAN 1956
ad_2	Adenine requiring (red)	276/3br	B. EPHRUSSI	EPHRUSSI *et al.* 1949
ad_6	Adenine requiring (white)	1742A	H. ROMAN	ROMAN 1956
ad_8	Adenine requiring (white)	A15–55	H. ROMAN	
ar_4	Arginine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
CU_1	Copper resistance		original isolate (DCH)	HAWTHORNE 1955a; BRENNES-POMALES *et al.* 1955; LINDEGREN *et al.* 1959
FI	Film formation		original isolate (DCH)	HAWTHORNE 1955a; MAGNI 1949
g_1	Galactose nonfermenter	1426A	C. C. LINDEGREN	LINDEGREN 1949; DOUGLAS and CONDIE 1954; HAWTHORNE 1956
g_2	Galactose nonfermenter	93–3P	C. C. LINDEGREN	DOUGLAS and CONDIE 1954; HAWTHORNE 1956
g_3 (g_8)	Galactose nonfermenter	186–2	O. WINGE	WINGE and ROBERTS 1948; HAWTHORNE 1956
g_4	Galactose nonfermenter	3352	A. P. JAMES	
hi_1	Histidine requiring	1914	C. RAUT	LINDEGREN and LINDEGREN 1951; LINDEGREN *et al.* 1959
hi_2	Histidine requiring		original isolate (DCH)	HAWTHORNE 1955a
hi_4	Histidine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
hi_6	Histidine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
is_1	Isoleucine requiring		original isolate (RKM)	MORTIMER *et al.* 1957; ROMAN and JACOB 1958; LINDEGREN *et al.* 1959

TABLE 1—(Continued)

The sources of the genes included in this report

Gene symbol	Phenotype	Strain	Received from	Reference
le$_1$	Leucine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
ly$_1$	Lysine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
ly$_2$	Lysine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
MA$_3$	Maltose fermenter		original isolate (DCH)	HAWTHORNE 1955a
MEL	Melibiose fermenter	CXL–10	C. C. LINDEGREN	LINDEGREN 1949; HAWTHORNE 1955a
me$_1$	Methionine requiring	67	S. POMPER	POMPER and BURKHOLDER 1949
me$_2$	Methionine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
p$_1$	Cytochrome deficient	B–26	B. EPHRUSSI	CHEN *et al.* 1950
p$_2$	Cytochrome deficient		original isolate (RKM)	MORTIMER *et al.* 1957
s-me$_1$	Suppresses *me*$_1$		original isolate (DCH)	
SU$_1$	Sucrose fermenter	93–3P	C. C. LINDEGREN	LINDEGREN 1949; HAWTHORNE 1955a
thr$_1$	Threonine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
thr$_2$	Threonine plus methionine or homoserine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
thr$_3$	Threonine plus methionine or homoserine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
thr$_4$	Threonine requiring		original isolate (RKM)	MORTIMER *et al.* 1957
tr$_1$	Tryptophane requiring	62–20–194	S. POMPER	POMPER and BURKHOLDER; HAWTHORNE 1955b
tr$_2$ *(an)*	Tryptophane requiring	1914	C. RAUT	LINDEGREN and LINDEGREN 1951; LINDEGREN *et al.* 1959
tr$_5$	Tryptophane requiring		original isolate (RKM)	MORTIMER *et al.* 1957
ur$_1$	Uracil requiring	62–20–194	S. POMPER	POMPER and BURKHOLDER 1949
ur$_3$	Uracil requiring		original isolate (RKM)	MORTIMER *et al.* 1957; LINDEGREN and LINDEGREN 1951; LINDEGREN *et al.* 1959

spored asci were dissected (HAWTHORNE 1955b). After the discovery of several centromere markers which simplified the analysis of unordered tetrads, it was more expeditious to utilize one of the various sodium acetate sporulation media (STANTIAL 1935; ADAMS 1949; FOWELL 1952), which yield principally oval (unordered) asci. A recent improvement in dissection technique has resulted from the use of snail digestive juices to digest the ascus wall (JOHNSTON and MORTIMER 1959). Only the data from complete tetrads are reported in this paper.

Nutritional requirements were assayed on synthetic media based on the one proposed by WICKERHAM (1946). Copper resistance was scored on the synthetic medium containing 1 mM/l. of copper sulfate (BRENNES-POMALES, LINDEGREN and LINDEGREN 1955). Fermentation tests were conducted in Durham tubes, in Kahn tubes, with brom-cresol-purple as a *p*H indicator, or on agar (two percent) with brom-thymol-blue as the indicator. The medium in each case was one percent peptone, one percent yeast extract, and one percent of the diagnostic sugar. The mating type of auxotrophic segregants was determined by the production of diploid prototrophs in crosses to haploids of known mating type and with complementary nutritional requirements. Otherwise, the determinations were based on the direct observation of zygotic figures. It was possible to score two or more complementary genes controlling either the fermentation of a sugar or a nutritional requirement using techniques described previously (HAWTHORNE 1956; ROMAN 1956).

Centromere linkage: Tetrad analysis of four-spored asci of Saccharomyces hybrids makes possible the location of a gene with respect to the centromere of its chromosome. Since centromeres of homologous chromosomes segregate at the first division of meiosis, a gene closely linked to the centromere will be expected to show a high frequency of first division segregation. The frequency of second division segregation is, therefore, a measure of the recombination frequency between the gene and the centromere. For genes closely linked to the centromere, the recombination frequency is one half the frequency of second division segregation. For genes with a greater interval, the recombination frequency must be calculated from equations which deal with the effects of multiple crossovers (BARRATT, NEWMEYER, PERKINS and GARNJOBST 1954).

When the relationship of the spores in the ascus is known, gene-centromere linkage can be determined directly. If the relationship of the spores cannot be ascertained from their position in the ascus and if known centromere markers are absent, it is necessary to use an indirect method for the estimation of the second division segregation frequency (LINDEGREN 1949; PERKINS 1949; and WHITEHOUSE 1950).

The asci from Saccharomyces hybrids are of two morphological types: (1) oval asci in which spore relationship is undeterminable, and (2) elongated asci in which the spores are arranged in a linear series. An investigation of the four-spored linear asci obtained from a diploid hybrid revealed that spores with non-sister nuclei alternate in the elongated ascus (HAWTHORNE 1955b). This conclusion was based primarily on data concerning the segregation of a single gene, tr_1, controlling the requirement for tryptophane. The spore arrays for trypto-

phane independence in 74 asci were as follows: 1(+ + − −), 1(+ − − +), 2(− + + −), and 70(+ − + −). The relegation of 70 out of 74 asci to a single spore array was interpreted as evidence that the fourth array, (+ − + −), is the noncrossover class and that the gene tr_1 is closely linked to the centromere. On this basis, the gene tr_1 was mapped at 2.5 units from the centromere since about five percent of the asci were in the second division segregation classes: (+ + − −), (+ − − +), and (− + + −).

Further dissections of linear asci provided evidence for nuclear slippage in some of the asci. The compilation of the spore array data for tr_1 from 515 asci is as follows: 9(+ + − −), 39(+ − − +), 28(− + + −), and 439(+ − + −). If the noncrossover class is strictly confined to the fourth array, the number of asci with the first array (+ + − − or − − + +) should be equal to the total number of asci with the second and third arrays. The unexpectedly high proportion of asci with the second and third arrays suggests that in five to ten percent of the asci, slippage has occurred and the sister nuclei are located together in the center and at the ends of the elongated ascus; some of the second and third arrays should therefore be included in the noncrossover class.

The error in the scoring of the asci for second division segregation, introduced by the occasional slippage of the nuclei may be contended with in two ways: (1) The frequency of asci with the first array, (+ + − −), may be used to obtain an estimate of second division segregation, since this array would be obtained in one half the cases of second division segregation whether slippage occurs or not. (2) A correction of the scoring of individual asci with evidence of nuclear slippage is also possible when a decision can be based on several independently segregating genes with close centromere linkage (HOWE 1956). For example, if in a given ascus each of the genes tr_1, ad_1, and g_1 (1, 5, and 8 units from their respective centromeres) segregated as either (+ − − +) or (− + + −), it would be safe to assume that these arrays represent the noncrossover class for this ascus since the probability of simultaneous second division segregation followed by a central-terminal assortment of these three loci is 0.00004.

The second procedure was the one generally followed for the determination of second division segregation. The data on the distribution of the spore arrays in linear asci from crosses in which it was possible to correct for the slippage of the nuclei are given in Table 2. Since this method of determining the first division segregation pattern in individual asci can also be applied to the unordered tetrads of oval asci, it was no longer necessary to dissect linear asci when the crosses involved several centromere markers. The data from oval asci on the segregation of the centromere linked genes are given in Table 3.

The genes listed in Tables 2 and 3 are divided in three groups on the basis of their second division segregation (SDS) frequencies: (1) SDS frequencies significantly less than 66.7 percent, (2) SDS frequencies about 66.7 percent, (3) SDS frequencies significantly higher than 66.7 percent. Centromere linkage is indicated by an SDS frequency that is significantly less than 66.7 percent. An SDS frequency of 66.7 percent is the upper limit predicted with no interference and unlimited crossing over between the locus and the centromere (MATHER

TABLE 2

Centromere linkage data from linear asci, corrected spore arrays

Locus	++−−	+−−+	−++−	+−+−	Total	Percent second div. segregation
g_3	1	1	0	83	85	2.4
tr_1	6	4	3	412	425	3.1
ad_1	13	7	10	230	260	11.5
g_1	27	14	11	256	308	16.9
FI	45	21	16	329	411	19.9
α	70	48	35	365	518	29.5
hi_2	39	21	28	147	235	37.5
MA_3	101	50	50	134	335	60.0
hi_1	74	46	44	91	255	64.4
MEL	149	60	74	126	409	69.2
ur_1	120	69	51	107	347	69.3
tr_2	121	59	39	86	305	71.8
me_1	151	68	69	103	391	73.7
g_2	57	50	31	45	183	75.4
ad_2	105	46	31	55	237	76.9

1935). Therefore, the SDS frequencies significantly higher than 66.7 percent are interpreted as evidence for chiasma interference and, moreover, indicate that the genes in question are close enough to the centromere for the effects of interference to be manifested. An SDS frequency in the neighborhood of 66.7 percent may mean either that the gene is located so far from the centromere that interference does not prevent the occurrence of multiple crossovers or that the gene is linked to the centromere by an interval of about 36 to 41 units (see Figure 3).

An examination of Tables 2 and 3 shows that there are 19 genes close enough to the centromere to be of value in this study; the degree of linkage ranges from one unit (tr_1, g_3) to 33 units (me_2). The centromere-linked genes were included in crosses in various combinations to determine the number of independent linkage groups and, thus, the number of chromosomes.

Linkage relationships of the centromere-linked genes: For the determination of a minimal chromosome number on the basis of the number of centromere markers, methods must be adopted for establishing nonlinkage as well as for detecting linkage. By establishing nonlinkage we mean a demonstration that two genes are not linked to the same centromere. With tetrad data obtained from a diploid of composition *A B/a b*, the statistics to be employed are the frequencies of parental ditype (PD) asci (*AB AB ab ab*), nonparental ditype (NPD) asci (*Ab Ab aB aB*), and tetratype (T) asci (*AB Ab aB ab*). The following criteria were adopted: (1) Linkage is indicated when the PD:NPD ratio is significantly in excess of 1:1, provided the prospective map distance between two centromere-linked genes is equal to either the sum or the difference of the gene-to-centromere distances. (2) Nonlinkage is indicated when the NPD:T ratio exceeds 1:4 (PERKINS 1953) or

if the recombination value for the genes under consideration is greater than the sum of the gene-to-centromere distances.

Consistent map distances are essential for the demonstration of linkage since occasionally tetrad ratios indicative of both linkage and nonlinkage are encountered. There are two phenomena which may give rise to this situation: (1) A preferential segregation of non-homologous centromeres or affinity (MICHIE and WALLACE 1953) can give a false indication of linkage. (2) Chromatid interference across the centromere resulting in an excess of four-strand double crossovers can give a false indication of nonlinkage.

When dealing with genes showing only slight centromere linkage, one may obtain tetrad ratios which are not indicative of either linkage or nonlinkage. However, some information can still be gained under these circumstances if, on the basis of consistent map distances, the possibility of locating the genes on the same chromosome arm can be eliminated. Examples illustrating these conditions and the resolution of the problems will be given below.

TABLE 3

Centromere linkage data from oval asci

Locus	First-division segregation	Second-division segregation	Percent second-division segregation
tr_1	895	4	0.45
le_1	850	39	3.9
ur_3	153	16	9.5
ad_1	629	70	10.0
p_1	293	34	10.4
g_1	454	82	15.3
ar_4	449	85	15.9
hi_2	332	151	31.3
tr_5	132	61	31.9
hi_6	207	100	32.5
α	650	432	39.9
hi_4	152	110	42.0
thr_1	189	144	42.3
ly_1	71	85	54.5
ad_6	175	234	57.3
g_4	68	92	57.5
me_2	110	163	59.7
ly_2	82	134	62.0
SU_1	103	169	62.2
ad_8	92	166	64.5
thr_3	76	143	65.4
MA_3	166	337	67.0
me_1	94	209	69.0
g_2	85	237	73.5
MEL	149	415	73.5
CU_1	71	224	76.0

In Table 4, there is presented a summary of all tetrad analyses which are indicative of linkages involving centromere-linked genes. Of the 19 centromere-linked genes (Tables 2 and 3), 15 show linkage either with one another or with genes not directly established as centromere-linked. There are nine genes in the latter category with SDS frequencies near 66.7 percent and higher. For the genes not listed in Tables 2 or 3, the SDS frequencies that were observed in samples of about 50 asci are as follows: *thr*$_4$ 58 percent, *s-me*$_1$ 67 percent, *is*$_1$ 69 percent, and *p*$_2$ 74 percent. The determination of gene sequence and map distances will be deferred until later in this paper.

Throughout this study, crosses were made specifically to gather evidence for nonlinkage for those centromere markers which appeared to be segregating independently. The first cross in this series gave diploids heterozygous for the centromere markers *tr*$_1$, *g*$_1$, *FI*, *hi*$_2$, and *α*, as well as *MA*$_3$, *MEL*, *ur*$_1$, *me*$_1$, and *ad*$_2$. Table 5 gives the data from 75 tetrads which had 2:2 segregations for all five of the centromere-linked genes. (Tetrads with irregular segregations, i.e., segregations deviating from the expected 2:2 ratio, were observed for most of the loci with a frequency on the order of one to two percent. To maintain a homogeneous sample, these asci are not included in the presentation of the segregation data for individual hybrids, Tables 5 through 9. However, data from these asci on the loci with 2:2 segregations were utilized in the determination of centromere linkage and the analysis of multiple crossovers, Tables 2, 3, 12, and 13.) Nonlinkage for each of the centromere-linked genes, *tr*$_1$, *g*$_1$, *FI*, *α*, and *hi*$_2$, was established with

TABLE 4

Linkage data for the centromere-linked genes

Gene pair	PD	NPD	T
g$_1$–*ly*$_2$	43	6	95
hi$_6$–*ly*$_1$	75	8	130
me$_2$–*p*$_2$	50	0	12
α–*hi*$_4$	97	7	174
α–*thr*$_4$	25	0	12
tr$_1$–*g*$_3$	70	0	0
tr$_1$–*s-me*$_1$	17	1	32
le$_1$–*tr*$_5$	226	0	61
le$_1$–*ad*$_6$	125	6	175
tr$_5$–*ad*$_6$	32	12	62
p$_1$–*ar*$_4$	163	0	18
ar$_4$–*thr*$_1$	213	0	62
p$_1$–*thr*$_1$	119	0	52
thr$_1$–*CU*$_1$	106	0	111
p$_1$–*CU*$_1$	60	8	164
ur$_3$–*thr*$_3$	64	1	105
thr$_3$–*hi*$_1$	64	0	1
hi–*is*$_1$	49	0	20
is$_1$–*tr*$_2$	56	0	13
hi$_1$–*tr*$_2$	118	2	95

TABLE 5

*The analysis of hybrids No. 1111 and No. 1113: data from 75 tetrads with 2:2 segregations for all the genes in question**

	tr_1	g_1	FI	α	hi_2	MA_3
tr_1		36:26 13	34:34 17	32:28 15	35:23 27	17:12 46
g_1	8.9 43.3		29:21 25	27:28 20	24:22 29	12:16 47
FI	11.8 43.3	17.9 44.7		27:29 19	21:20 34	12:12 51
α	10.3 47.3	16.4 50.7	19.3 51.3		21:18 36	11:15 49
hi_2	18.9 48.7	25.0 48.7	27.9 49.3	26.4 48.0		9:19 47
MA_3	34.0 46.7	40.1 52.7	43.0 50.0	41.5 52.7	50.1 56.7	
Number second div. seg.	2	11	15	13	25	46
Percent second div. seg.	2.7	14.7	20.0	17.4	33.3	61.2
Centromere distance $k = 0.1$	1.4	7.5	10.4	8.9	17.5	32.6

* In the upper portion of the grid, the numbers of parental (PD) and nonparental (NPD) ditypes and the tetratypes (T) are given as $\frac{\text{PD:NPD}}{\text{T}}$ for the various combinations. In the lower portion of the grid, the summed centromere distances of the two genes being compared are given over the observed recombination values.

the observation that the NPD:T ratios for each pair was significantly greater than 1:4. The comparison of the summed centromere distances with the recombination values supported this conclusion. In every case, the recombination value (a minimal representation of the distance between the loci in the case of linkage) greatly exceeded the sum of the centromere distances.

From the second cross, a hybrid was obtained which was heterozygous for the following genes: *tr*$_1$, *ad*$_1$, *g*$_1$, *FI*, α, *hi*$_1$, *MA*$_3$, *SU*$_5$, *MG*$_2$ (alpha-methyl glucoside fermentation), *s-me*$_1$, *ur*$_1$, *MEL*, *ad*$_2$, and *can* (canavanine resistance). An analysis of the data from 50 tetrads, Table 6, supports the independent segregation of the centromere markers, *tr*$_1$, *ad*$_1$, *g*$_1$, *FI*, and α. Linkage between *tr*$_1$, and *s-me*$_1$ was indicated by a $\frac{\text{PD:NPD}}{\text{T}}$ ratio of $\frac{17:1}{32}$. Since there was no crossing over between *tr*$_1$ and the centromere in these tetrads, the one NPD ascus indicates a single four-strand double exchange occurred in the interval between *s-me*$_1$ and the centromere. This result gave promise that linkage between a gene with a similar SDS frequency (64 percent) and any of the markers showing strong centro-

TABLE 6

*The analysis of hybrid No. 1249: data from 50 tetrads with 2:2 segregations for all the genes in question**

	tr_1	ad_1	g_1	FI	α	hi_1	MA_3
tr_1		24:17 9	21:20 9	20:23 7	13:19 18	10:11 29	11:7 32
ad_1	9.3 43.0		23:11 16	13:22 15	9:15 26	13:5 32	8:6 36
g_1	9.3 49.0	18.6 38.0		13:14 23	14:17 19	10:10 30	9:8 33
FI	7.2 53.0	16.5 59.0	16.5 51.0		13:15 22	13:10 27	7:11 32
α	19.0 56.0	28.3 56.0	28.3 53.0	26.2 52.0		10:10 30	9:14 27
hi_1	31.0 51.0	40.3 42.0	40.3 50.0	38.2 47.0	50.0 50.0		14:9 27
MA_3	34.8 46.0	44.1 48.0	44.1 49.0	42.0 54.0	53.8 55.0	65.8 45.0	
Number second div. seg.	0	9	9	7	18	29	32
Percent second div. seg.	0	18	18	14	36	58	64
Centromere distance $k = 0.1$	0	9.3	9.3	7.2	19.0	31.0	34.8

* See footnote, Table 5.

mere linkage, tr_1, le_1, etc., through ar_4, would not go undetected. Therefore, the genes hi_1 and MA_3, considered to be loosely linked to the centromere, are included in Table 6. The PD:NPD ratio of 13:5 for hi_1-ad_1 is suggestive of linkage; however, subsequent investigations have shown that hi_1 and ad_1 are not linked and that hi_1 is located on the chromosome marked by ur_3 (later in this paper). Of the remaining genes, MA_3, SU_5, and MG_2 are closely linked (no crossovers in this sample of 50 asci) while the others segregate independently from each other and the centromere markers.

Two strains differing in the centromere-linked genes tr_1, le_1, ad_1, p_1, g_1, ar_4, hi_2, tr_5, α, hi_4, thr_1, and ad_6 were crossed to give hybrid X714, Table 7. Irregular segregations for the ad_1 locus, with the tetrads distributed as 57 (4:0), 9 (3:1), and 31 (2:2), suggested that the hybrid was trisomic for the chromosome carrying this gene (therefore, AD_1 AD_1/ad_1). None of the remaining centromere markers or the other genes present, MA_3, ad_8, g_2, CU_1, and MEL, gave irregular

ratios indicative of location on this chromosome. The assortment of the centromere markers tr_1, le_1, p_1, g_1, hi_2, and either α or hi_4 met the criteria for non-linkage. The $\frac{23:5}{55}$ ratio for α and hi_4 is a significant demonstration of linkage. From a comparison of the recombination value (38) with the centromere distances for α (20 units) and hi_4 (24 units), the genes were located on the opposite sides of the centromere.

Of the remaining centromere markers, tr_5 and ad_6 are linked to le_1; and ar_4, thr_1, together with CU_1, are linked to p_1 (Table 4). Following an examination of the crossover patterns in individual asci, the sequence tr_5, le_1, centromere, and ad_6 was adopted (later in this paper) while the genes p_1, ar_4, thr_1, and CU_1 were placed in that order on the same chromosome arm. Although the distal gene of the latter group, CU_1 showed 70 percent SDS in this cross, only a single four-strand

TABLE 7

*The analysis of hybrid X714: data from 81 tetrads with 2:2 segregations for all the genes in question**

	g_1	α	hi_4	tr_1	hi_2	le_1	p_1
g_1		23:22 / 36	20:23 / 38	28:40 / 13	18:33 / 30	36:31 / 14	40:27 / 14
α	29.7 / 49.3		23:3 / 55	24:23 / 34	16:28 / 37	23:25 / 33	19:25 / 37
hi_4	33.0 / 51.8	46.3 / 37.8		22:20 / 39	14:18 / 49	17:27 / 37	17:23 / 41
tr_1	8.8 / 57.4	22.1 / 49.3	25.4 / 48.8		33:27 / 21	39:37 / 5	41:35 / 5
hi_2	21.1 / 59.2	34.4 / 57.4	37.7 / 52.5	13.5 / 46.3		35:27 / 19	22:35 / 24
le_1	10.7 / 47.0	24.0 / 51.2	27.3 / 56.1	3.1 / 48.8	15.4 / 45.0		35:38 / 8
p_1	10.7 / 42.0	24.0 / 53.7	27.3 / 53.7	3.1 / 46.3	15.4 / 58.0	5.0 / 51.8	
Number second div. seg.	13	33	38	1	20	4	4
Percent second div. seg.	16.0	40.7	46.9	1.2	24.7	4.9	4.9
Centromere distance $k = 0.1$	8.2	21.5	24.8	0.6	12.9	2.5	2.5

* See footnote, Table 5.

TABLE 8

*The analysis of hybrid No. 1295: data from 50 tetrads with 2:2 segregations for all the genes in question**

	tr_1	le_1	p_1	ad_1	g_1	α	hi_2
tr_1		22:25 / 3	40:8 / 2	26:18 / 6	21:23 / 6	18:11 / 21	15:21 / 14
le_1	3.0 / 53.0		20:26 / 4	27:16 / 7	14:27 / 9	13:13 / 24	17:18 / 15
p_1	2.0 / 18.0	5.0 / 56.0		27:16 / 7	20:22 / 8	17:10 / 23	15:19 / 16
ad_1	6.1 / 42.0	9.1 / 39.0	8.1 / 39.0		20:18 / 12	9:14 / 27	15:17 / 18
g_1	6.1 / 52.0	9.1 / 63.0	8.1 / 52.0	12.2 / 48.0		15:13 / 22	17:15 / 18
α	22.2 / 43.0	25.2 / 50.0	24.2 / 43.0	28.3 / 55.0	28.3 / 48.0		17:9 / 24
hi_2	14.6 / 56.0	17.6 / 51.0	16.6 / 54.0	20.7 / 52.0	20.7 / 48.0	36.8 / 42.0	
Number second div. seg.	0	3	2	6	6	21	14
Percent second div. seg.	0	6	4	12	12	42	28
Centromere distance $k = 0.1$	0	3	2	6.1	6.1	22.2	14.6

* See footnote, Table 5.

double crossover and four other multiple exchanges were observed in the centromere-CU_1 interval. In contrast, a $\frac{28:12}{53}$ ratio for tr_5-ad_6 reflects a surprisingly high frequency of four-strand double exchanges occurring in this region spanning the centromere; four-strand exchanges account for more than half of the double crossovers observed (Table 12).

The observation of a barely significant PD:NPD ratio, 28:12, for tr_5-ad_6 gave warning that with the customary analysis one might miss detecting linkage between two genes similarly situated but a little more loosely linked to the centromere. A more sensitive analysis to detect trans-centromere linkage utilizes only the tetrads which have first division segregations for both of the genes under consideration (CATCHESIDE 1951). Under the hypothesis that the genes in question are on the opposing arms of the same chromosome: (1) all noncrossover tetrads must be of the first division segregation class, and (2) all NPD tetrads showing first division segregation must have resulted from a four-strand double crossover within one of the gene-centromere intervals. For example, if, with the above data,

only the ditype asci showing first division segregation are examined, the new PD:NPD ratio for tr_5-ad_6, 21:0, is highly significant evidence for linkage.

With the fourth cross in this series, there was obtained a hybrid, No. 1295, yielding asci with 2:2 segregations for the following centromere markers for the seven chromosomes predicted with the previous cross: tr_1, p_1, le_1, ad_1, g_1, hi_2, and α. The data from 50 tetrads presented in Table 8 supports the interpretation of nonlinkage for these genes. Regular segregations were observed for these additional markers: ar_4, thr_1, me_2, SU_1, ad_8, ur_2, ur_1, ad_3, MEL, MA_3, and CU_1. Again as shown in the previous cross, the genes p_1, ar_4, thr_1, and CU_1 proved to be linked. The rest of the genes segregated independently from each other and the centromere markers.

TABLE 9

*The analysis of hybrid X709: data from 65 tetrads with 2:2 segregations for all the genes in question**

	ad_1	g_1	α	tr_1	hi_2	le_1	hi_6	ar_4	ur_3
ad_1		27:24 / 14	15:18 / 32	26:30 / 9	19:21 / 32	33:20 / 12	14:22 / 29	21:28 / 16	30:27 / 8
g_1	11.0 / 47.6		11:21 / 27	26:32 / 7	22:19 / 24	30:24 / 11	20:20 / 25	21:29 / 15	33:22 / 10
α	25.8 / 52.3	24.2 / 53.1		12:29 / 24	15:16 / 34	23:14 / 28	11:16 / 38	14:20 / 31	17:20 / 28
tr_1	7.0 / 53.1	5.4 / 54.6	20.2 / 63.0		21:19 / 25	26:33 / 6	17:26 / 22	29:25 / 11	31:29 / 3
hi_2	25.8 / 49.4	24.2 / 47.6	39.0 / 50.8	20.2 / 48.4		21:14 / 30	15:19 / 31	16:19 / 30	20:17 / 28
le_1	10.2 / 40.0	8.6 / 45.3	23.4 / 43.1	4.6 / 55.4	23.4 / 44.6		20:21 / 24	19:31 / 15	25:34 / 6
hi_6	23.2 / 56.1	21.6 / 50.0	36.4 / 53.8	17.6 / 57.0	36.4 / 53.1	20.8 / 50.8		17:19 / 29	19:23 / 23
ar_4	14.2 / 55.4	12.6 / 56.2	27.4 / 54.5	8.6 / 46.9	27.4 / 52.3	11.8 / 59.2	24.8 / 51.5		23:28 / 14
ur_3	9.4 / 47.6	7.8 / 41.5	22.6 / 52.3	3.8 / 48.4	22.6 / 47.7	7.0 / 56.9	20.0 / 53.1	11.0 / 53.8	
Number second div. seg.	8	6	24	1	24	5	21	10	4
Percent second div. seg.	12.3	9.2	37.0	1.5	37.0	7.7	32.3	15.4	6.2
Centromere distance $k = 0.1$	6.3	4.7	19.5	0.7	19.5	3.9	16.9	7.9	3.1

* See footnote, Table 5.

The tetrad ratio observed for p_1-tr_1 $\frac{(40:8)}{2}$ is an anomaly incompatible with either independent segregation or linkage. Since nonlinkage between tr_1 and p_1 was clearly indicated by the analysis of hybrid X714, this result was interpreted as evidence for the preferential segregation of non-homologous centromeres. One other PD:NPD ratio, 14:27 for le_1-g_1, differed significantly, at the five percent level, from 1:1; however, from the 21 combinations of the seven centromere markers listed in Table 8, one chance deviation from 1:1 at this level of significance is not surprising.

The last cross in this series provided evidence for at least nine different chromosomes in Saccharomyces. The analysis of 65 tetrads from hybrid X709, Table 9, shows that two new centromere markers ur_3 and hi_6 segregated independently from the following genes marking the seven centromeres definitely established with the previous cross: tr_1, le_1, g_1, ad_1, ar_4, hi_2 and α.

Four more genes hi_4, thr_3, g_2, and *MEL* were segregating in the asci of hybrid X709. Further evidence for linkage between hi_4 and α was obtained with a $\frac{24:2}{39}$ ratio for this gene pair. Linkage between thr_3 and ur_3 was indicated by a $\frac{28:0}{37}$ ratio. There was no suggestion of linkage between g_2 and *MEL* and any of the other markers.

There was no evidence of preferential segregation of non-homologous centromeres in this cross. In contrast to the results obtained in the preceding cross, the centromeres marked by tr_1 and p_1 (in this case ar_4) segregated as 1:1 for parental and nonparental combinations. In Table 9, there is one PD:NPD ratio that does deviate significantly from 1:1 at a one percent level, the ratio of 12:29 for tr_1–α. However, the PD:NPD ratio of 15:21 for tr_1 and hi_4, located across the centromere from α, is not significant. By utilizing the results with both hi_4 and α to determine the segregation of this centromere relative to the one marked by tr_1, a PD:NPD ratio of 25:37 is obtained which again is not significant evidence for the preferential segregation of these centromeres.

Two groups of genes remain to be considered: (1) those genes closely linked to a gene already established as centromere linked will be dealt with in the next section; (2) those genes showing so little centromere linkage as to have been of little use in the early phases of this problem will be considered below. The genes in the latter category have SDS frequencies ranging from 54.5 percent for ly_1 to 64.5 percent for ad_8 (see Table 3). In some cases, the numbers of tetrads analyzed are insufficient to demonstrate a significant deviation from the 66.7 percent SDS expected with no centromere linkage. Even a significant deviation from 66.7 percent SDS might not necessarily be conclusive evidence for centromere linkage since it is conceivable that a SDS frequency in this range might be the consequence of a pronounced chromatid interference favoring two-strand or four-strand double crossovers.

TABLE 10

Tetrad analysis of the data from miscellaneous crosses involving the centromere markers and the genes showing slight centromere linkage

	ad_1	g_1	α	tr_1	ur_3	hi_2	le_1	p_1	hi_6
ly_1	19:26 / 53	13:18 / 37	48:86 / 187	10:16 / 39		4:4 / 10	22:17 / 43	10:13* / 22	75:8 / 130
ly_2	6:12 / 33	43:6 / 95	31:33 / 115	20:22 / 70	5:4 / 21	11:12 / 65	42:23 / 115	37:17 / 81	
g_4	10:15 / 37	11:8 / 29	16:13 / 87	41:25 / 90	10:11 / 33	14:15 / 50	19:20 / 78	14:10 / 25	7:11 / 36
me_2	21:24 / 90	27:23 / 83	42:42 / 153	37:47 / 128	11:16 / 41	23:20 / 63	19:19 / 82	24:30 / 92	9:13 / 42
SU_1	26:24 / 81	25:23 / 82	37:43 / 167	33:29 / 92	13:7 / 28	15:14 / 63	32:34 / 117	39:24 / 104	17:17 / 63
MA_3	49:45 / 171	45:48 / 230	68:57 / 261	38:34 / 172	10:7 / 37	35:32 / 125	48:42 / 203	15:21 / 79	34:25 / 129
ad_8	13:26 / 74	26:31 / 110	43:49 / 161	42:42 / 150	15:8 / 30	34:30 / 110	28:25 / 111	42:33 / 133	3:3 / 12

* The gene ar_4 marked the centromere of chromosome VIII in the analysis with ly_1.

† The numbers of parental (PD) and nonparental (NPD) ditypes and the tetratypes are given as $\frac{\text{PD:NPD}}{\text{T}}$.

Table 10 gives the data concerning the segregation of these genes of questionable centromere linkage, ly_1, g_4, me_2, ly_2, SU_1, MA_3, and ad_8 with respect to the centromere markers of the nine chromosomes established with hybrid X709. Two cases of linkage are evident: ly_1–hi_6 $\frac{75:8}{130}$ and ly_2–g_1 $\frac{43:6}{95}$. A $\frac{41:25}{90}$ ratio for g_4–tr_1 differs significantly from 1:1. However linkage between g_4 and tr_1 is unlikely, provided g_4 is actually centromere linked, since this ratio would require four-strand double crossovers greatly in excess of the numbers expected on the basis of the frequency observed for what would be a comparable region, tr_1–*s*-me_1 (see the discussion of hybrid 1249). The tetrad ratios show that me_2 is segregating independently from the nine centromere markers. Further evidence supporting centromere linkage for me_2 and thus indicating nonlinkage with these nine centromere markers will be given later. The three loci with a little more tenuous indication of centromere linkage, SU_1, MA_3, and ad_8, are also segregating independently from these nine centromere markers.

In a further attempt to locate these new markers, an analysis was made of their segregations with respect to each other and the following genes which show a similar order of centromere linkage: ly_2 chromosome II, hi_1 chromosome V, ad_6 chromosome VII, CU_1 chromosome VIII, and ly_1 chromosome IX. If one accepts these genes as being centromere linked, it is readily apparent from the data in

Table 11 that they all must be located on different chromosome arms. The one $\frac{\text{PD:NPD}}{\text{T}}$ ratio indicative of linkage, $\frac{23:11}{72}$ for g_4–CU_1 is attributed to the statistical fluctuation expected in a table of these dimensions since g_4 showed no link-

TABLE 11

Tetrad analysis of the data from miscellaneous crosses involving the genes showing slight centromere linkage

	g_4	SU_1	ad_8	MA_3	ly_2	hi_1	ad_6	CU_1	ly_1
me_2	30:21 / 81	15:20 / 91	17:21 / 54	38:37 / 136	18:22 / 55	8:7 / 29	11:9 / 39	23:33 / 107	3:3 / 28
g_4		10:7 / 46	19:12 / 50	9:13 / 54	7:4 / 35	5:8 / 14	15:17 / 56	23:11 / 72	8:4 / 12
SU_1			17:9 / 61	19:28 / 77	6:7 / 26	15:8 / 44	7:11 / 41	14:21 / 75	2:9 / 33
ad_8				25:28 / 108	1:5 / 14	1:2 / 15	25:23 / 100	30:28 / 114	
MA_3					11:9 / 58	28:25 / 67	10:11 / 44	20:14 / 87	24:18 / 50

* The numbers of parental (PD) and nonparental (NPD) ditypes and the tetratypes are given as $\frac{\text{PD:NPD}}{\text{T}}$.

TABLE 12

Multiple crossovers in regions spanning the centromere

Linkage group	Distribution of tetrads		Distribution of double crossovers: 2:3:4-strand double exchanges	Expected double crossovers k = 1.0	coincidence
III	no c.o.	85			
	c.o. I	75			
I II	c.o. II	75			
hi_4–c–α	c.o. I and II	43	12:24:7	50	0.86
		278			
VII					
I II III	no c.o.	21			
tr_5–leu_1–c–ad_6	c.o. I	6			
	c.o. II	2			
	c.o. III	41			
	c.o. I and II	0			
	c.o. I and III	20	6:4:10	21.3	1.08
	c.o. II and III	3	1:0:2		
		93			
IX	no c.o.	67			
	c.o. I	36			
I II	c.o. II	83			
hi_6–c–ly_1	c.o. I and II	27	8:11:8	32.5	0.83
		213			

TABLE 13

Crossovers in adjacent regions of linkage group VIII

I II III IV
c–p_1–ar_4–thr_1–CU_1

Distribution of tetrads*		Distribution of double crossovers:
no c.o.	33	2:3:4-strand double exchanges
c.o. I	5	4:3:2 + 1 (2 or 4 strands)
c.o. II	13	
c.o. III	21	
c.o. IV	63	
c.o. I and III	1	
c.o. I and IV	1	
c.o. III and IV	6	
c.o. II, III and IV	1	
	144	

Region	Crossovers	Map length	Locus	Centromere distance	Second div. segregation Number	Second div. segregation Percent
I	7	2.4	p_1	2.4	7	4.8
II	14	4.9	ar_4	7.3	21	14.6
III	29	10.0	thr_1	17.3	47	32.6
IV	71	24.7	CU_1	42.0	105	72.9

* Data from hybrids 1295 and X714.

age to the centromere marker of chromosome VIII, p_1 (see Table 10). The PD:NPD ratios for me_2–g_4, g_4–ad_8, and ad_8–SU_1 deviated enough from 1:1 to warrant an analysis of the first division segregation tetrads to check for trans-centromere linkage. The restricted PD:NPD ratios for me_2–g_4 (13:11), g_4–ad_8 (11:6), and ad_8–SU_1 (5:4) did not support this possibility of linkage. Thus, the establishment of nonlinkage for me_2, g_4, SU_1, MA_3, ad_8 and the centromere markers of the first nine chromosomes depends only upon the verification of centromere linkage for these markers. To date the evidence for centromere linkage justifies only the inclusion of me_2 on the chromosome maps as the centromere marker of chromosome X.

Chromosome maps: The results of the individual crosses presented in the preceding section can now be summarized by the construction of chromosome maps, Figure 1. Ten chromosomes are included in these maps; their centromeres are marked by the genes ad_1, g_1, α, tr_1, ur_3, hi_2, le_1, p_1, hi_6, and me_2. All genes established as being linked to the above centromere markers are represented on the maps. Distances were computed using the mapping functions of BARRATT *et al.* (1954), Figure 3. Except for the loci on chromosome VIII, the calculations are based on the data presented in Tables 2, 3, and 4. For the mapping of the loci on chromosome VIII, a more homogeneous sample, the data obtained from hybrids 1295 and X714 (Table 13) is used. The procedure followed to determine gene-centromere sequence will be illustrated below in the placement of the centromere in the linkage group tr_5–le_1–ad_6.

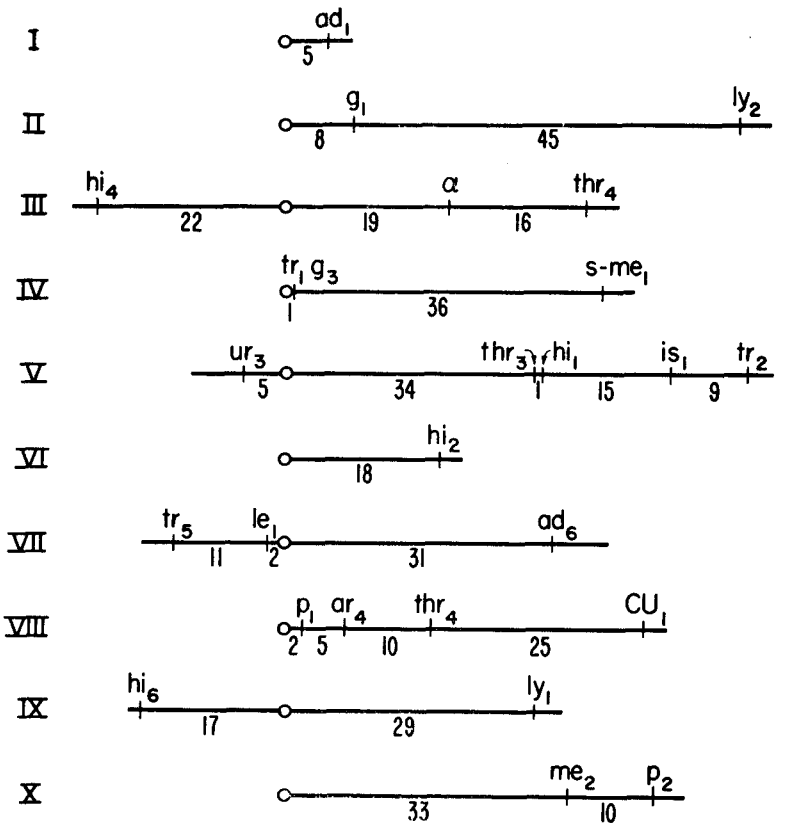

FIGURE 1.—Chromosome maps of Saccharomyces. The map distances are derived from the second division segregation frequencies in Tables 2 and 3 and the tetrad ratios in Table 4.

The three genes, tr_5, le_1, and ad_6 gave 2:2 segregation in 93 of the tetrads analyzed in the third cross. Among these tetrads, there were five with second division segregations for le_1. The centromere segregation in these five tetrads was determined by the segregation of the genes tr_1, p_1, and g_1 so there was no doubt as to the occurrence of a crossover between le_1 and the centromere. Figure 2 gives the segregation patterns for the three loci and the crossover configurations that are needed under the hypotheses that the centromere is located (1) between le_1 and ad_6 and (2) between tr_5 and le_1. Only three segregation patterns were observed in these asci. Two asci are represented by the array in the first row. To obtain this segregation of the three loci, two crossovers would be required under the first hypothesis compared to four under the second. There were two asci with the second array, and again the first hypothesis is favored; only one crossover needs to be postulated if the centromere is between le_1 and ad_6, while three are required if it is between tr_5 and le_1. In the ascus with the last array, two crossovers are required under either hypothesis. On the basis of these results, the sequence tr_5–le_1–c–ad_6 was adopted (c indicates centromere).

The sequences c–g_1–ly_2 chromosome II, ur_3–c–thr_3 chromosome V, c–p_1–ar_4 chromosome VIII, and hi_6–c–ly_1 chromosome IX were determined from similar analyses. A comparison of the SDS frequencies and recombination values was sufficient to order the loci hi_4–c–α–thr_4 on chromosome III and c–me_2–p_2 on chromosome X. However, support for the latter order and the centromere linkage of me_2 came from an examination of the asci that were tetratype for me_2–p_2 which showed that for the most part me_2 had segregated in the first division as would be expected with the expression of chiasma interference as demonstrated in chromosome VIII (see later in this paper). The arrangement of the genes on chromosome IV, c–tr_1–g_3–s-me_1, is provisional since no asci with a crossover between g_3 and tr_1 have been recovered and only first division segregations have been observed for tr_1 in the crosses involving g_3 and s-me_1 (see Table 4).

An analysis of the crossover pattern in individual tetrads was also needed to establish the sequence of the closely linked genes on chromosome V. The order of thr_3 and hi_1 with respect to the centromere or the other markers was not determined until the analysis of data on 40 asci from a hybrid heterozygous for ur_3, thr_3, hi, and tr_2, made available through the courtesy of Dr. F. Sherman, gave us a tetratype tetrad for thr_3 and hi_1. In this ascus parental ditype segregations were observed for ur_3–thr_3 and hi_1–tr_2 thus clearly favoring the order ur_3–thr_3–hi_1–tr_2. The summary of the tetrad ratios for is_1 with hi_1 and tr_2 (Table 4) includes data from the work of Roman and Jacob (1958). A crossover analysis as well as a comparison of the recombination values places is_1 between hi_1 and tr_2.

Interference and the distribution of multiple crossovers: Preliminary to the selection of a mapping function, information was sought on the occurrence of chiasma or chromatid interference for both regions spanning the centromere and those restricted to one arm. Data on the crossovers in three regions in the first

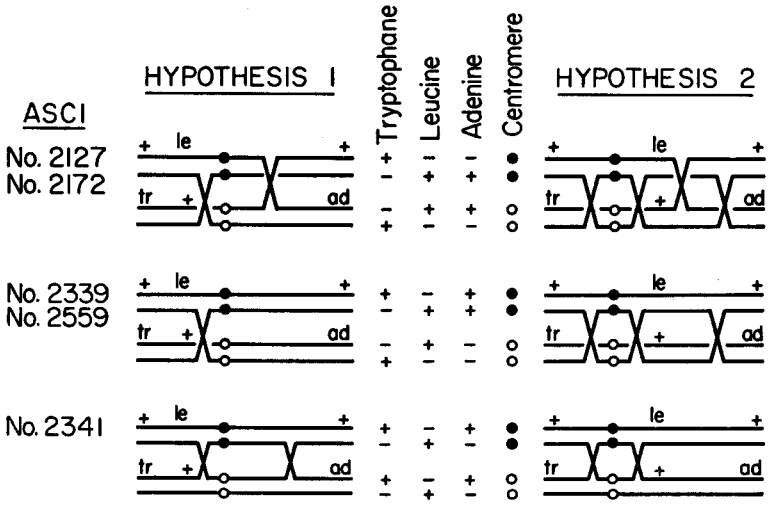

Figure 2.—Examples of the segregation patterns for tr_5, le_1, ad_6, and the centromere, showing the consequences of the alternative positions of le_1 with respect to the centromere.

category, chromosome III α–c–hi_4, chromosome VII tr_5–le_1–c–ad_6, and chromosome IX hi_6–c–ly_1, are presented in Table 12. None of the three regions gave convincing evidence of chiasma interference for crossovers in opposing arms. Ratios of observed to expected double crossovers of 43/50, 23/21.3 and 27/32.5 were obtained for the gene–centromere–gene intervals of chromosomes III, VII, and IX, respectively. All double crossovers were classified as to two-strand, three-strand or 4-strand exchanges to detect any indication of chromatid interference, *i.e.*, any deviation from the 1:2:1 ratio of two-, three-, and four-strand doubles expected on the classical model of crossing over. For chromosome III, the double crossovers are distributed as 12 two-strand, 24 3-strand, and 7 four-strand, in agreement with the 1:2:1 ratio. For chromosome VII, the 7:4:12 distribution is a significant departure from the 1:2:1 ratio expected. The 8:11:8 distribution for chromosome IX is again compatible with the 1:2:1 ratio. The summed frequencies for the three chromosomes, 27 2-strand, 39 3-strand, and 27 four-strand, fit satisfactorily the frequencies expected with the 1:2:1 distribution predicted with no chromatid interference.

Among the linkage groups on one arm, the loci c–p_1–arg_4–thr_1–CU_1 embraced the only region that was long enough to give information on the occurrence of double crossovers, yet contained markers spaced so that the chance of a double exchange occurring within an interval was negligible. Data from the analyses of hybrids 1295 and X714 on the crossovers which occurred within this region are presented in Table 13. There were 144 tetrads available where all the genes in question gave 2:2 segregations. The numbers of crossovers observed in the four intervals progressing from the centromere were 7, 14, 29, and 71. If, with the locus thr_1, we divide this region into two, the effects of chiasma interference are readily apparent. The ratio of observed to expected number of asci having crossovers in both 'halves' was 8/24. A more precise evaluation of the degree of chiasma interference was obtained through the following analysis of the data. The asci were classified according to tetrad rank or the number of crossovers for the entire region (WEINSTEIN 1936). In the 144 asci, there were 33 tetrads of rank 0 (no exchange), 102 of rank 1 (a single exchange), eight of rank 2, and one of rank 3. For no chiasma interference, the relative frequency of tetrads of different rank can be predicted from the Poisson distribution (BARRATT *et al.* 1954). Corresponding to the observed frequency of the tetrads of rank 0 (33 per 144 asci), one would expect 49 tetrads of rank 1, 35 of rank 2, 18 of rank 3, and nine of ranks higher than 3. The lack of agreement between the observed distribution and the numbers expected, if the crossovers occurred independently, indicates the influence of a strong positive chiasma interference. To obtain a useful expression of the degree of chiasma interference, use was made of the relation derived by BARRATT *et al.* 1954, (equation 7) to estimate tetrad rank frequency for different degrees of chiasma interference. In this relationship, the degree of interference is specified by the value of a coefficient 'k'. For no interference, k=1. For complete interference, k=0. Since a high degree of chiasma interference was indicated, tetrad rank distributions were calculated for k=0.2 and k=0.1. Corre-

sponding to the 33 rank 0 tetrads observed and with k=0.2, one would expect 94 tetrads of rank 1, 14 of rank 2, two of rank 3 and one of ranks higher than 3; while for k=0.1, the expected distribution is 103 tetrads of rank 1, 7.5 of rank 2, and 0.5 of rank 3. It can be seen that the values predicted with k=0.1 fit very closely the observed frequencies in all the ranks.

Evidence for some variation in the degree of interference for the above region in chromosome VIII came from the analysis of 56 asci from miscellaneous crosses which in some cases were lacking the markers p_1 or ar_4. The summed data, with a distribution of ten tetrads of rank 0, 39 of rank 1, and seven of rank 2, were indicative of a lesser degree of interference than that observed with hybrids 1295 and X714.

The choice or deriviation of a mapping function requires a knowledge regarding the strand relationships in multiple exchanges, i.e., the occurrence of chromatid interference, as well as the extent of chiasma interference. The data concerning multiple exchanges in one arm are again confined to the c–p_1–ar_4–thr_1–CU_1 region. In the above analyses, the multiple exchanges were distributed as six two-strand, five three-strand, and three two- or four-strand. Although this distribution conforms more closely to a 1:1:1 ratio, it is not a significant deviation from the 1:2:1 ratio expected with no chromatid interference. This distinction poses no problems when mapping centromere linked genes. However, for mapping loci with SDS frequencies significantly higher than 67 percent, a distinction between the two distributions becomes of importance due to the accumulation of multiple exchanges. Curves of the SDS frequencies based on both the 1:1:1 and 1:2:1 ratios of two-strand, three-strand, and four-strand exchanges for k=0.1 and k=0.2 are given in Figure 3. The frequency of NPD asci are also plotted for the four cases. By selecting the interference level and strand distribution which best fits the observed $\frac{\text{PD:NPD}}{\text{T}}$ ratio, one can obtain an estimate of the map distance between loosely linked genes.

DISCUSSION

Of the 140 mutant loci now in our breeding stocks about half have been investigated for centromere linkage. Twenty-seven genes had SDS frequencies of less than 67 percent (Tables 2 and 3). Twenty-two of these genes can be placed in ten different linkage groups (Figure 1). Only two of the remaining genes, *FI* and g_4, gave SDS frequencies that deviated significantly from 67 percent. Satisfactory scoring for the character film formation was obtained only in the highly inbred stocks used in the earlier experiments; for this reason the gene *FI* has not been included in the final maps. Although it is likely that g_4 marks the centromere of another chromosome, it is not presently included in the chromosome maps since the criteria for nonlinkage has not been satisfied for all the genes marking the centromeres of the first ten chromosomes, Table 10. With the accumulation of more tetrads showing the segregation of SU_1, ad_8, and MA_8, it is expected that

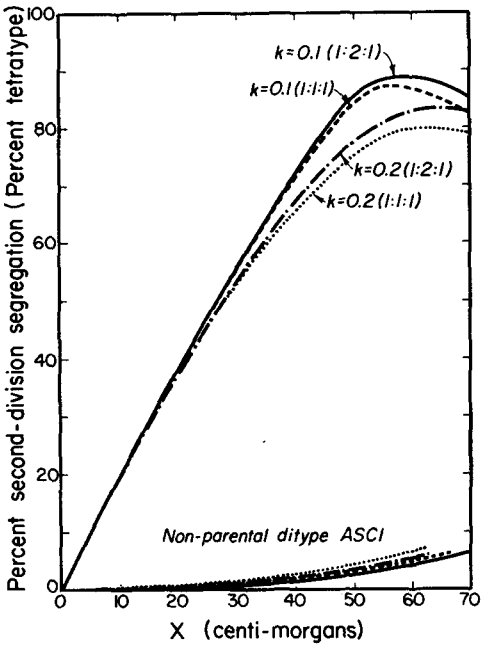

FIGURE 3.—Tetrad mapping functions (based on the derivation of BARRATT, NEWMEYER, PERKINS, and GARNJOBST 1954). Percent tetratype and nonparental ditype asci as a function of map distance for different degrees of chiasma and chromatid interference. The degree of chiasma interference is indicated by the value of k($k = 1.0$, no interference, $k = 0$, complete interference). Chromatid interference is represented by the distribution of two-strand, three-strand, and four-strand multiple exchanges (in parentheses).

some of these genes will prove to mark the centromeres of additional chromosomes.

In the designation of the chromosomes, an attempt was made to follow the numbering of the chromosomes listed by LINDEGREN and LINDEGREN (1951). They present maps of five chromosomes with the following centromere markers: (I) *ad-1*, (II) *g*, (III) *α*, (IV) *pb* (paraminobenzoic acid), and (V) *ur*. However, the use of the gene *pb* to mark the centromere of chromosome IV is not justified from the analysis of the published data (Table 24–3, LINDEGREN 1949), since, as PERKINS (1953) has pointed out, the $\frac{\text{PD:NPD}}{\text{T}}$ ratio of $\frac{17:5}{45}$ for *pb–α* is actually indicative of linkage at a 99 percent level of significance. The four remaining chromosomes can be recognized. The genes marking the centromeres of chromosomes I, II, and III are common to our stocks: ad_1 was received from DR. E. L. TATUM and *g* (*g-1*) and *α* were received from DR. C. C. LINDEGREN. The uracil gene marking chromosome V was presumed to be ur_3 because of similar linkage relationships with hi_1 and the centromere (LINDEGREN *et al.* 1959). For chromosome IV, we have chosen the linkage group with the centromere markers tr_1 and

g_3. The remaining centromere markers designate chromosomes in the order in which independent assortment was established.

In a recent summary of linkage studies by LINDEGREN, LINDEGREN, SHULT, and DESBOROUGH (1959), there are listed seven and possibly nine linkage groups associated with centromeres. No data were furnished concerning the centromere linkage of the markers identifying these linkage groups: *CH* (choline), *UR*, *TH* (thiamine), *CU*, *GA*, *α*, *AD*, *PR* (proline), and *DX* (dextrin fermentation). However, four of the chromosomes correspond to linkage groups I, II, III and V in this study and in the earlier maps presented by LINDEGREN and LINDEGREN (1951) although their designation has been revised. We have decided to adhere to the original designations for reasons of priority and because of the rather speculative nature of their latest maps. For example, 'chromosome I' marked by *CH*, *UR*, and *TH* is presented as either a chromosome with three branches or a complex of chromosomes with absolute affinity of parental centromere combinations.

The centromere distances indicated in the earlier reports of LINDEGREN and LINDEGREN (1949, 1951) for the markers ad_1 11 units, g_1 six units, *α* 24 units, and *ur* five units agree well with the averages of our own determinations: ad_1 five units, g_1 eight units, *α* 19 units, and ur_3 five units. No attempt has been made to present confidence limits for the gene centromere intervals since for some genes, there appears to be considerable variation from cross to cross. The most exaggerated variation was observed for the mating type locus which showed only slight centromere linkage in the earlier crosses—58 percent SDS for the 74 tetrads of hybrids 1002, 1003, and 1004 (HAWTHORNE 1955b)—but was more closely linked in the hybrids of this study 17.4 percent SDS hybrids 1111 and 1113, 36 percent SDS hybrid 1249, 42 percent SDS hybrid 1295, 40 percent SDS hybrid X714, and 37 percent SDS hybrid X709. Two genes that have been established as being more loosely linked to their centromeres, hi_1 and CU_1, have also shown a significant variation in SDS frequency. With these loci the SDS frequencies have varied from 55 percent to 75 percent.

The above variation in SDS frequencies may be a reflection of changes in the interference constant as well as the map distance. An indication of this for the c–thr_1–CU_1 region of chromosome VIII came from the analysis of multiple exchanges in asci from miscellaneous crosses where the interference was less pronounced than that observed for hybrids X714 and 1295. Moreover the very strong interference demonstrated for chromosome VIII in hybrids X714 and 1295 is not necessarily applicable to the other chromosomes. For instance, with chromosome II, the $\frac{43:6}{95}$ ratio for g_1–ly_2, 62 percent SDS for ly_2, and the order c–g_1–ly_2 cannot be reconciled with a strong chiasma interference and a 1:2:1 distribution of two-strand, three-strand, and four-strand double exchanges. Since changing the postulated sequence of the loci to g_1–c–ly_2 requires nearly a doubling of the number of multiple exchanges between g_1 and ly_2, it seems more probable that it is the interference constant which is at variance with that observed for chromosome VIII.

SUMMARY

Extensive linkage studies in Saccharomyces were undertaken with more than 70 genes controlling nutritional requirements, fermentation of sugars, resistance to metals, and colonial characters. The emphasis was on the comparison of centromere linked markers, detected through tetrad analysis, to obtain an estimation of the minimal haploid chromosome number. Seventeen genes that showed a significant degree of centromere linkage were compared and found to fall into ten linkage groups which segregated independently. Another nine genes showing significant linkage to the centromere markers were placed on the maps of these chromosomes. There were five more genes with at least a suggestion of centromere linkage (a second division segregation frequency less than 67 percent) which remain unmapped.

An analysis of chiasma and chromatid interference was made for four of the linkage groups. Chiasma intereference was not observed in three groups comprised of regions spanning the centromere, while a coincidence value of 0.34 was observed for the linkage group with regions restricted to a single arm. For the latter group, as well as one of the three groups encompassing the centromere, the frequency of two-strand and four-strand double crossovers was greater than expected on the basis of a 1:2:1 distribution of two- three-, and four-strand multiple exchanges.

An analysis of five hybrids heterozygous for centromere markers on as many as nine chromosomes revealed only one exception to the independent assortment of the chromosomes. In this one hybrid, a single pair of non-homologous centromeres segregated preferentially in the parental combination.

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